AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

EPIDEMIOLOGY

# Diagnostic Performance of Multiplex Nucleic Acid Testing of Bronchoalveolar Lavage and Bronchial Wash Specimens for Respiratory Viral Pathogens

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**ABSTRACT** There is limited knowledge on the yield of performing multiplex nucleic acid testing (NAT) on multiple lower respiratory tract specimens from a single patient with a single instance of infection. We evaluated the performance characteristics of multiplex NAT assays performed concurrently on bronchoalveolar lavage (BAL) and bronchial wash (BW) specimens to detect respiratory pathogens. A retrospective study of admitted patients from March 2013 through December 2016 was performed. Individual performance characteristics of BAL and BW specimens were compared to positive results from either set of specimens. Only contemporaneous BAL and BW specimens (received by the laboratory within 4 h of each other) were included. The final cohort included 170 patients, with 184 contemporaneous BAL and BW specimens submitted for multiplex NAT (median age, 58 years; 62% male). Of the patients with positive NAT results, 38 of 40 BW specimens tested positive (overall percent agreement with combined testing, 98.9%; 95% confidence interval [CI], 95.5 to 98.9%), and 34 of 40 BAL specimens tested positive (overall percent agreement with combined testing, 96.7%; 95% CI, 93.0 to 96.7%). Assays performed on BW specimens identified 4 additional specimens and had a higher positive percent agreement (95.0%) with combined testing results compared to those performed on BAL specimens (85.0%). There was exact concordance in 174 specimens (94.6%; negative and positive for respiratory pathogens, 144 and 34 specimens, respectively). We observed high concordance (95%) between multiplex NAT results from contemporaneous BAL and BW specimens. Performance characteristics of BW specimen testing were equivalent to those of BAL specimen testing. The benefit of performing additional testing should be carefully considered against the potential complications and health care costs.

**KEYWORDS** multiplex, respiratory viruses, virus testing

Respiratory viruses are a common cause of upper respiratory tract infection and are responsible annually for approximately 200 million cases of pneumonia worldwide (1). Accurate diagnosis of respiratory viral pathogens is necessary to identify patients who benefit from specific treatments (such as antivirals), limit unnecessary antimicrobial use, and prevent nosocomial transmission by employing appropriate transmission-based infection control precautions (1–7).

Previously, viruses were detected in respiratory specimens using direct fluorescence antibody assays, rapid antigen detection immunoassays, or viral culture. However, studies have reported that these tests have low sensitivity (8–12). With the advent of nucleic acid testing (NAT), the ability to diagnose respiratory viral infections and characterize their epidemiology has greatly improved (13). A number of commercially

**Received** 14 June 2018 **Returned for modification** 11 July 2018 **Accepted** 6 August 2018

**Accepted manuscript posted online** 9 August 2018

**Citation** Munigala S, Burnham C-AD, Anderson NW, Liang SY, Lawrence SJ, Warren DK. 2018. Diagnostic performance of multiplex nucleic acid testing of bronchoalveolar lavage and bronchial wash specimens for respiratory viral pathogens. J Clin Microbiol 56:e00973-18. https://doi.org/10.1128/JCM.00973-18.

**Editor** Daniel J. Diekema, University of Iowa College of Medicine



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available multiplex NAT assays have been developed to simultaneously detect multiple respiratory pathogens within a single specimen.

The performance characteristics of various tests to detect respiratory pathogens may differ based upon specimen type (14–16). For example, using older testing methods, nasopharyngeal wash was superior to nasopharyngeal or oropharyngeal swabs at detecting respiratory syncytial virus by indirect fluorescent antibody test and rapid enzyme immunoassay (16). More recent studies have compared multiplex NAT results between nasopharyngeal versus bronchoalveolar lavage (BAL) specimens from the same patient and obtained within 7 days (21, 22) or during the same procedure (23). The reported concordance between assay results from nasopharyngeal and BAL specimens in the above studies ranged from 77% to 89%. However, there is limited knowledge on the relative performance characteristics of multiplex NAT for different lower respiratory tract specimens, specifically for BAL versus bronchial wash specimens, or whether target detection is improved when multiple lower respiratory tract specimens are obtained from a single patient. These data have the potential to reduce health care costs by eliminating unnecessary testing.

The purpose of this study is to compare the relative diagnostic yield of multiplex NAT for respiratory viruses performed on bronchoalveolar lavage specimens versus bronchial wash specimens.

## MATERIALS AND METHODS

**Setting.** This was a retrospective cohort study. Using the hospital medical informatics database for microbiological and clinical data, we evaluated all patients admitted to Barnes-Jewish Hospital (BJH), a tertiary care academic medical center, from March 2013 through December 2016, who had a multiplex NAT respiratory pathogen test performed on both BAL and bronchial wash specimen.

Multiplex NAT respiratory pathogen testing was implemented at BJH on 4 March 2013. The assay used was the FilmArray respiratory panel (RP; BioFire Diagnostics, Salt Lake City, UT), used per the manufacturer's instruction. Respiratory viruses included on this panel include rhinovirus/enterovirus, influenza (A, A/H1, A/H3, A/H1N1-2009, and B), parainfluenza, respiratory syncytial virus (RSV), human metapneumovirus, coronavirus, and adenovirus. Results of tests for bacterial pathogens using this assay were not evaluated in this study. Tests performed on nasopharyngeal, nasal wash, and sputum specimens were not evaluated in this study.

**Selection of study population.** For each admitted patient, we matched multiplex NAT results from BAL and bronchial wash specimens using specimen collection time. To account for potential clerical differences in the time when specimens from the same procedure were collected, we defined contemporaneous BAL and bronchial wash testing as specimens marked as being collected within 4 h of each other (i.e., consistent with being obtained during a single bronchoscopy procedure). A pair of BAL and bronchial wash specimens collected within 4 h of each other was considered a single observation for this study. If a patient had multiple bronchoscopies during an admission in which contemporaneous BAL and bronchial wash specimens were obtained and NAT was performed with each bronchoscopy, then each pair of BAL and bronchial wash specimens was considered a distinct observation.

**Bronchoalveolar lavage and bronchial washing.** At our institution, a volume of 50- to 60-ml aliquots of normal saline is instilled during bronchoscopy for BAL procedure. The saline is instilled only after the bronchoscope is in a wedged position (i.e., cannot advance any further). This can be repeated subsequently 2 to 3 times, with the total volume of instilled fluid not exceeding 150 to 180 ml. The first 50 to 60 ml of instilled saline is aspirated back into a container. If this first aspirate is not discarded and sent for testing, it is described as a "bronchial washing." Most often the second (and sometimes third) instilled saline aliquots, which are called BAL specimens, are aspirated and sent for the analysis.

Separate low-volume washes from a proximal airway, which are more commonly used for pulmonary toileting, are excluded from the analysis (Fig. 1).

**Statistical analyses.** Patient demographics, ward location, length of hospital stay, comorbidities, and discharge disposition were extracted from the hospital's medical informatics database. The proportion of positive tests was determined. Since there is no established gold standard, performance characteristics of multiplex respiratory pathogen NAT on BAL or bronchial wash specimens were individually compared with the combined positive test results for BAL or bronchial wash specimens (i.e., if either the BAL or bronchial wash specimen was positive by NAT, then the combined test was considered positive), using a $2 \times 2$ table. Overall agreement and percent positive and percent negative agreement with 95% confidence intervals were calculated (see http://statpages.info/ctab2x2.html). Data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC).

This study was approved by the Washington University Human Research Protection Office with a waiver of consent.

## RESULTS

**Study cohort.** We identified 3,434 multiplex NAT respiratory pathogen tests performed on BAL and bronchial wash specimens during the study period. Patients who

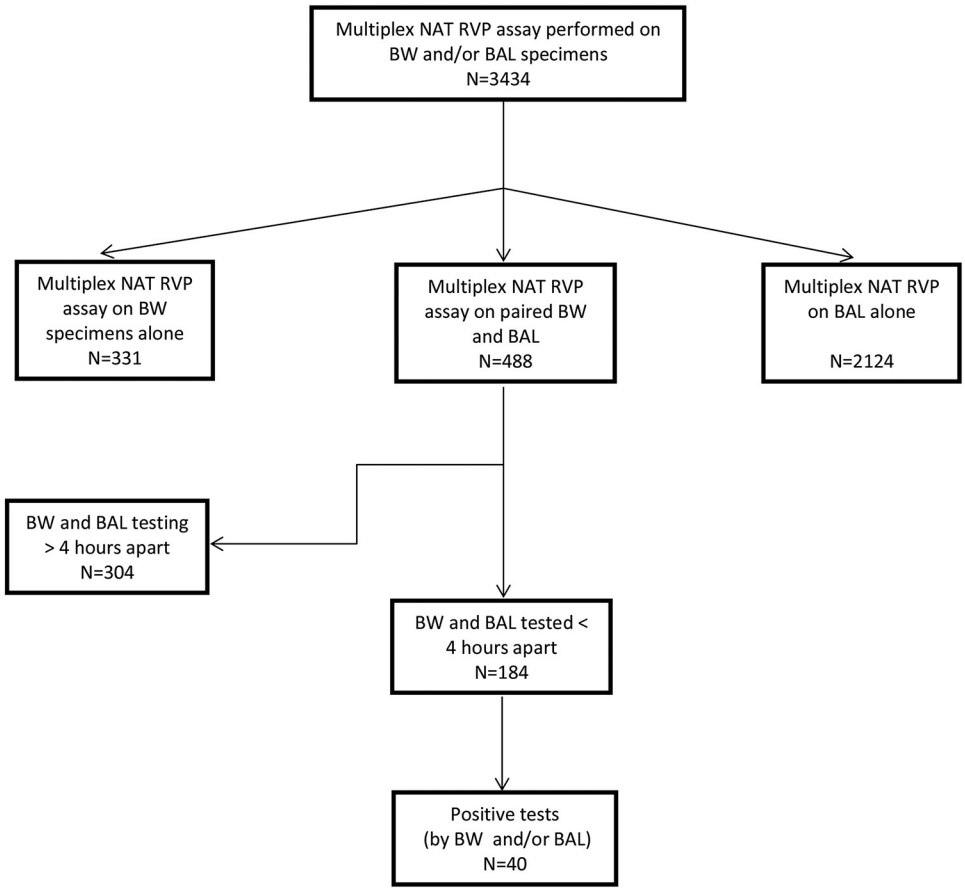


**FIG 1** Study cohort. NAT, nucleic acid testing; RVP, respiratory virus pathogen; BAL, bronchoalveolar lavage; BW, bronchial wash.

had BAL and bronchial wash testing >4 h apart ($n = 304$) and those who had testing on BAL specimens only ($n = 2,124$) or testing on bronchial wash specimens only ($n = 331$) were excluded. After exclusions, the final study cohort included 184 paired BAL and bronchial wash tests from 170 patients (Fig. 1). Nine patients (5.3%) had more than one test per admission. The median age of the patients was 58 years, 62% were male, and about 71% were white. A majority of the tests were performed within an intensive care unit (ICU) (66.3%). Approximately 57% of patients had pneumonia, 37.5% had acute respiratory failure, 34.2% had chronic obstructive pulmonary disease (COPD), 9.2% had acute bronchitis, and 1.6% had a history of lung transplant. A total of 40 of 184 (21.7%) bronchoscopy procedures resulted in a positive test result by BAL or bronchial wash specimen (median age, 58; 57.5% male; 77.5% white; 45% of these tests were performed within an ICU) (Table 1). Patients with a positive result were more likely to be ICU patients. The most common respiratory pathogens recovered from either specimen type were rhinovirus/enterovirus (20 of 40 patients [50.0%]), followed by influenza A (7 of 40 patients [17.5%]) and coronavirus (4 of 40 patients [10.0%]).

**Performance characteristics.** Comparing individual specimen type results to combined results for both specimens, 38 (95%) of the 40 patients with any positive specimen had a positive bronchial wash specimen. The concordance of bronchial wash results and combined specimen test results was 98.9% (95% confidence interval [CI], 95.5 to 98.9%). A total of 34 (85%) patients with any positive specimen had a positive BAL specimen. Percent agreement of BAL specimen tests with combined test results was 96.7% (95% CI, 93.0 to 96.7%). Compared to BAL testing, bronchial wash testing identified four additional positive specimens and had a significantly higher positive

**TABLE 1** Demographic characteristics of 184 patients with contemporaneous, paired lower respiratory tract specimens tested by multiplex nucleic acid test, by result for viral pathogen[a]

| Demographic characteristic[b] | All tests ($N = 184$) | Tests with: Any positive result ($N = 40$) | Tests with: Negative result ($N = 144$) | *P* value[c] |
|---|---|---|---|---|
| Age in yrs (median, IQR) | 58 (44–65) | 58 (44–66) | 58 (44–66) | 0.692 |
| Female gender | 70 (38.0) | 17 (42.5) | 53 (36.8) | 0.512 |
| Race | | | | |
| White | 130 (70.7) | 31 (77.5) | 99 (68.8) | Ref |
| African American | 42 (22.8) | 9 (22.5) | 33 (22.9) | 0.747 |
| Other | 12 (6.5) | | 12 (8.3) | 0.976 |
| Location | | | | |
| ICU | 122 (66.3) | 18 (45.0) | 104 (72.2) | 0.011 |
| Medicine | 31 (16.8) | 11 (27.5) | 20 (13.9) | Ref |
| Surgery | 15 (8.2) | 5 (12.5) | 10 (6.9) | 0.886 |
| BMT/oncology | 14 (7.6) | 5 (12.5) | 9 (6.3) | 0.988 |
| Cardiology | 2 (1.1) | 1 (2.5) | 1 (0.7) | 0.683 |
| Length of hospital stay in days (median [IQR]) | 18 (11–36) | 20 (11–35) | 18 (11–37) | 0.612 |
| Comorbidities | | | | |
| Pneumonia | 105 (57.1) | 25 (62.5) | 80 (55.6) | 0.433 |
| Acute respiratory failure | 69 (37.5) | 19 (47.5) | 50 (34.7) | 0.14 |
| Chronic obstructive pulmonary disease | 63 (34.2) | 13 (32.5) | 50 (34.7) | 0.793 |
| Acute bronchitis | 17 (9.2) | 6 (15.0) | 11 (7.6) | 0.155 |
| Acute respiratory distress syndrome | 7 (3.8) | 1 (2.5) | 6 (4.2) | 1.00 |
| Acute respiratory infections | 7 (3.8) | 3 (7.5) | 4 (2.8) | 0.176 |
| Lung transplant | 3 (1.6) | | 3 (2.1) | |
| Discharge status | | | | |
| Skilled nursing facility/other hospital | 24 (13.1) | 2 (5.0) | 22 (15.3) | Ref |
| Home | 119 (64.7) | 25 (62.5) | 94 (65.3) | 0.165 |
| Expired | 41 (22.3) | 13 (32.5) | 28 (19.4) | 0.045 |

[a]Study cohort included 184 paired BAL and bronchial wash tests from 170 unique patients. Each pair of BAL and bronchial wash specimens collected within 4 h of each other was considered a single observation for this study. Demographic characteristics were reported for these 184 paired BAL and bronchial wash tests (contemporaneous specimens).
[b]IQR, interquartile range; ICU, intensive care unit; BMT, bone marrow transplant. Values are reported as *n* (%) unless otherwise noted.
[c]Ref, reference category.

percent agreement of 95.0% (95% CI, 87.1 to 95.0%) versus 85.0% for BAL testing (95% CI, 76.3 to 85.0%) (Table 2).

There was concordance between bronchial wash and BAL test results in 174 (94.6%) of the 184 specimen pairs. Of the 10 pairs with discordant test results, BAL tests were negative in 6 specimens, whereas bronchial wash testing was positive for influenza A in 1 specimen and for rhinovirus/enterovirus in 5 specimens. In 2 specimens, the bronchial wash specimen test was negative, whereas the BAL specimen test was positive (1 influenza A and 1 adenovirus). In the remaining two specimen, bronchial

**TABLE 2** Comparison of multiplex nucleic acid test results by specimen type

| Specimen type and results[b] | Bronchial wash or BAL specimen test results ($N = 184$)[a]: Positive ($N = 40$) | Bronchial wash or BAL specimen test results ($N = 184$)[a]: Negative ($N = 144$) | Overall agreement (% [95% CI]) | Positive % agreement (95% CI) | Negative % agreement (95% CI) |
|---|---|---|---|---|---|
| Bronchial wash | | | 98.9 (95.5–98.9) | 95.0 (87.1–95.0) | 100 (97.8–100) |
| Positive (*n* [%]) | 38 (20.6) | 0 (0.0) | | | |
| Negative (*n* [%]) | 2 (1.1) | 144 (78.3) | | | |
| BAL | | | 96.7 (93.0–96.7) | 85.0 (76.3–85.0) | 100 (97.6–100) |
| Positive (*n* [%]) | 34 (18.5) | 0 (0.0) | | | |
| Negative (*n* [%]) | 6 (3.3) | 144 (78.2) | | | |

[a]Any positive result noted for BAL and/or bronchial wash paired specimens was used as a gold standard.
[b]BAL, bronchoalveolar lavage; CI, confidence interval.

**TABLE 3** Performance of bronchial wash versus BAL specimen multiplex nucleic acid test, by pathogen[a]

| Pathogen tested by bronchial wash NAT | Pathogen tested by BAL NAT[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | None | Flu A | RSV | Adenovirus | Coronavirus | Metapneumovirus | Parainfluenza | Rhinovirus/ enterovirus | Total (bronchial wash) |
| None | **144** | 1[c] | 0 | 1[c] | 0 | 0 | 0 | 0 | 146 |
| Flu A | 1[c] | **5** | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| RSV | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | 1 |
| Adenovirus | 0 | 0 | 0 | **4** | 0 | 0 | 0 | 0 | 4 |
| Coronavirus | 0 | 0 | 0 | 0 | **4** | 0 | 0 | 1[d] | 5 |
| Metapneumovirus | 0 | 0 | 0 | 0 | 0 | **1** | 0 | 0 | 1 |
| Parainfluenza | 0 | 0 | 0 | 0 | 0 | 0 | **2** | 1[d] | 3 |
| Rhinovirus/Enterovirus | 5[c] | 0 | 0 | 0 | 0 | 0 | 0 | **13** | 18 |
| Total (BAL) | 150 | 6 | 1 | 5 | 4 | 1 | 2 | 15 | 184 |

[a]There were no influenza B viruses detected in the final cohort. NAT, nucleic acid test; BAL, bronchoalveolar lavage; RSV, respiratory syncytial virus; Flu A, influenza A.
[b]Numbers in bold are concordant.
[c]Discordant.
[d]Positive for different organisms. For two patients, bronchial washing testing detected two organisms (parainfluenza and rhinovirus/enterovirus for patient one and coronavirus and rhinovirus/enterovirus for patient 2) but bronchoalveolar lavage testing identified only one organism (rhino/enterovirus for both patients).

wash testing detected two pathogens (parainfluenza and rhinovirus/enterovirus for 1 specimen and coronavirus and rhinovirus/enterovirus for the other specimen), but the BAL specimen was positive only for rhinovirus/enterovirus in either specimen (Table 3).

## DISCUSSION

Accurate diagnosis of respiratory viral infections is necessary to identify patients who might benefit from specific treatments, such as antivirals, and to prevent nosocomial spread using appropriate transmission-based infection control precautions (1–7). Several studies have reported that multiplex NAT has a greater sensitivity than viral cultures for diagnosing respiratory viral infections (17, 18). The diagnostic yield of performing the multiplex NAT on multiple versus a single lower respiratory tract specimen is not well studied. Performing multiple assays on respiratory specimens from a single bronchoscopy may unnecessarily increase the cost of care. In our study, we identified 95% concordance between multiplex NAT by bronchial wash specimen and by BAL specimen for respiratory pathogens. Performance characteristics of testing by bronchial wash specimen were comparable to those of testing of contemporaneously obtained BAL specimens, identifying 4 additional patients in our cohort who were positive for viral pathogens.

Bronchoalveolar lavage is associated with potential risks, such as worsening oxygenation and hemodynamic instability in critically ill patients (19, 20); therefore, any additional benefit of performing BAL should be carefully weighed against the potential risk. In a recent study comparing the yield of BAL sample to that of nasopharyngeal (NP) swab using the FilmArray respiratory panel, in 17 out of 86 patients (20%), BAL identified pathogens that were not detected by NP swab. BAL was found to be useful after a negative NP swab but did not add any new microbiological information once the pathogen was identified by NP swab (21). Even in our study, although bronchial wash specimen testing identified 4 additional patients positive for viral respiratory pathogens, BAL testing identified two specimen pairs with positive BAL specimens but negative bronchial wash specimen, including one BAL specimen positive for influenza A. While the cost and risk of bronchoscopy may not necessarily be averted by equivalent detection of viruses in a bronchial wash (BW) specimen, studies should consider optimization of viral pathogen detection, while reducing cost of testing.

Limitations of our study include its single-center retrospective design. We did not conduct individual chart review for each patient. We did not have the data for intubation. Therefore, we are unable to separate out bronchoscopic BAL done in intubated patients versus nonintubated patients. We also did not have the ability to perform additional testing to resolve discrepant results. We assumed BAL and bronchial wash specimens with collection times documented within 4 h of each other to represent specimens collected from a single procedure, which could have introduced

bias into our assessment. However, the majority of these specimens (82%; $n = 150$) had identical collection times, and 92% ($n = 169$) of them had collection times within 30 min. Of the remaining 15 paired tests, 10 had collection times within 30 min to 2 h of each other, and 5 specimens had recorded collection times greater than 2 h apart. When we limited our analysis to only paired specimens collected within 30 min of each other, the results were unchanged (data not shown). Due to lack of a comparator assay, we used concordance and a positive result by either BAL or bronchial wash specimen testing as the comparator for this analysis. Furthermore, we did not evaluate the provider who performed the bronchoscopies, which may affect how each specimen was collected. Since the main reason for BAL in patients with suspected infection is to detect bacterial pathogens and not for viral detection, we compared the proportion of BAL versus BW specimens from our final cohort sent for additional testing for bacterial pathogens (aerobic Gram-negative bacteria, *Legionella* spp., and *Mycobacterium* spp.) and found no difference (data not shown). Strengths of our study include a large sample size, evaluation of specimens obtained over multiple influenza seasons, and use of a short window of comparison (4 h) in which the ability to detect virus is significantly higher.

In conclusion, our study shows that there is very high concordance between multiplex NAT performed on bronchial wash and BAL specimens for suspected respiratory virus infections. As the performance characteristics of bronchial wash specimens were comparable to those of BAL specimens, in instances where BAL has a low return volume, the first-aliquot "bronchial washing" specimen can be considered for viral PCR testing, while reducing the costs of multiple specimen testing. Future studies need to evaluate this concordance in a larger patient population.

## ACKNOWLEDGMENT

This work was supported by Washington University departmental funds issued to D.K.W. for the Hospital Epidemiology and Infection Prevention Program.

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